

Small but voracious: invasive generalist consumes more zooplankton in winter than native planktivore

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Abstract

In recent years, Lake Constance has experienced an invasion and domination of three-spined stickleback (*Gasterosteus aculeatus*) in the pelagic zone, which has coincided with a decline in the native whitefish (*Coregonus wartmanni*) population. Similar massive invasions of sticklebacks into pelagic zones have been recognized also in marine areas or small lakes worldwide. However, their diet overlaps with native species is rarely evaluated, especially in the winter season, which often presents a bottleneck for fish survival. In this study, we compared the diet of pelagic sticklebacks with the diet of the substantially larger native whitefish in different seasons, to evaluate the threat of the recent stickleback invasion on whitefish populations. By monthly sampling of zooplankton and both fish species diets, we could demonstrate that sticklebacks select similar prey throughout most of the year and consume more prey than whitefish during the winter. With relations between prey availability and prey selection, interspecific and intraspecific seasonal diet variability and indices like a prey-specific index of relative importance, we discuss the importance of zooplankton species traits and abundance for whitefish and stickleback predation. This study shows that sticklebacks, despite their small size, represent a serious potential diet competitor to native planktivorous fish. Sticklebacks quickly adapt to new environments, and thus we advocate precautions regarding their introduction into similar lakes as Lake Constance, as this could cause irreversible ecological changes.

Keywords

Bythotrephes longimanus, Lake Constance, overwintering strategy, pelagic whitefish diet, planktivory, predator size, seasonal prey selection, stickleback invasion

Introduction

The spread of invasive species can cause irreversible changes in ecosystems because it often affects many biological organisational levels, from genes to ecosystems (Ellender and Weyl 2014). Native species with an analogous ecological role can be especially affected if the invader is superior in resource utilisation (Dick et al. 2017). Dietary overlap is an important factor that can be used to predict the likelihood of competition when resources are limited (Sale 1974). Furthermore, understanding seasonal changes in the abundance of prey and the selection of prey by predators is fundamental for understanding the interactions between native and invasive species as well as energy transfer within ecosystems (Baxter et al. 2004). Therefore, studying the diets of invaders *in situ* and comparing them with natives is valuable for assessing possible ecological consequences.

Lake Constance is facing a new fish invasion, and besides a pilot study (Bretzel et al. 2021), no study to date has investigated the seasonal diet of the invader – the three-spined stickleback (*Gasterosteus aculeatus* Linnaeus, 1758; henceforth referred to as “stickleback”). Sticklebacks were first reported in the lake in the 1950s (Muckle 1972; Roch et al. 2018); however, for decades, their presence was limited to the littoral zone. Then, in 2012/2013, sticklebacks suddenly invaded the pelagic zone in high numbers (~2300 per ha) (Rösch et al. 2018; Eckmann and Engesser 2019). In September 2014, they represented more than 95% of the number and 25% of the biomass of all pelagic fish (Alexander and Vonlanthen 2016). Except for the Baltic Sea, which has brackish water, sticklebacks rarely appear in the pelagic zone in such abundance (Jakubavičiute et al. 2016). The impact of sticklebacks on freshwater zooplankton, which is by far the most important invertebrate food in the pelagic zone, is rarely investigated and mostly restricted to smaller shallow lakes (Campbell 1991; Sanchez-Gonzales et al. 2001; Jakobsen et al. 2003). Furthermore, the effects of increased predation pressure from sticklebacks on the diet of whitefish (*Coregonus wartmanni* Bloch, 1784), which was historically and until recently a keystone pelagic fish in Lake Constance (Eckmann and Rösch 1998) has not yet been studied.

Although Lake Constance is among the most studied lakes globally, the diet of whitefish was analysed only sporadically. The first study was done almost 100 years ago during the initial oligotrophic state of the lake (Auerbach et al. 1924; Elster 1944), while during the eutrophic state of the lake only two sampling campaigns of whitefish diet were made (Hartmann 1983; Becker and Eckmann 1992; Eckmann et al. 2002). Afterwards, the lake underwent re-oligotrophication, which decreased the yield of whitefish (Baer et al. 2016). In parallel with the invasion of sticklebacks, whitefish yield additionally declined and whitefish growth was further reduced (Rösch et al. 2018). Probably, these reductions are related to increased competition for food caused by the stickleback invasion; however, functional studies are lacking. Additionally, during the invasion, the zooplankton community underwent significant changes, e.g. a sudden increase in the numbers of a small daphniid, *Daphnia cucullata* (IGKB 2020), whose role in fish diets is unknown.

The final sizes of sticklebacks and whitefish greatly differ (Kottelat and Freyhof 2007). According to Kleiber’s law (Kleiber 1947), larger whitefish are expected to

consume more food per individual but less per biomass than smaller sticklebacks. As both fish species also differ in their spawning and juvenile growing seasons (Kottelat and Freyhof 2007), differences in their feeding behaviours and diet preferences might be more pronounced during certain seasons. As body size plays an important role in overwintering feeding strategies in some species (van Deurs et al. 2011), differences between both fish species could result in their predation differences in winter. Except for young and small fish, which are limited by their gape size (Hartmann 1983; Branstator and Lehman 1996; Makrakis et al. 2008), zooplanktivorous fish generally select larger, more conspicuous, and more abundant prey (Lazzaro 1987; Gliwicz and Pijanowska 1989). Laboratory data show that already 2-cm-long sticklebacks can consume prey in the same size range as larger and older first-year-of-life (0+) whitefish (Ogorelec et al. 2022). However, whitefish and sticklebacks have different sizes, morphologies, and feeding strategies, and thus their predation success on various types of zooplankton may differ *in situ*. Sticklebacks are small fish and considered feeding generalists, consuming a wide range of littoral and pelagic food (Wootton 1984). By contrast, the pelagic whitefish is a specialised zooplankton feeder with morphological and behavioural adaptations for effective zooplanktivory (Lazzaro 1987) and can selectively pick large quantities of the larger zooplankton species, e.g. *Bythotrephes longimanus* (Eckmann et al. 2002). Therefore, they might feed differently and more selectively than sticklebacks.

To better understand the diets and feeding relations of whitefish and sticklebacks *in situ*, we conducted a 1-year seasonal diet study, sampling fish using gillnetting and trawling, and assessing their stomach content. We aimed to assess 1) whether sticklebacks consume more zooplankton biomass per body weight than whitefish, 2) diet differences depending on prey availability and season, 3) whether zooplanktivorous whitefish are more selective than generalist sticklebacks, and 4) the implications of the zooplankton consumption of sticklebacks on whitefish.

Methods

Study site

Upper Lake Constance is located in the south of Germany and represents the main basin of Lake Constance. It is a lake with an area of 476 km² and an average depth of 101 m. With increased human population and eutrophication, the concentration of phosphorus peaked at around 90 µg/L in the late 1970s. Afterwards, building wastewater treatments and the phosphorus ban in detergents started to show effects on phosphorus concentration, which gradually decreased and in the 2000s approached values recorded in the early 1950s (below 10 µg/L). Nowadays, the lake is oligotrophic, average chlorophyll-a concentrations are around 2–3 µg/L, diatoms are the dominating algae, and the density of zooplankton is low (dry weight in the upper 20 m = 80 µg/L) (IGKB 2018). The lake contains around 30 fish species, among which only whitefish,

sticklebacks, perch (*Perca fluviatilis* Linnaeus, 1758), lake trout (*Salmo trutta* Linnaeus, 1758), arctic char (*Salvelinus* cf. *umbla* Linnaeus, 1758), and deepwater char (*Salvelinus profundus* Schillinger, 1901) occupy the pelagic zone in the adult stage of life (Eckmann et al. 2006; Alexander and Vonlanthen 2016).

Sampling

From April 2017 to May 2018, monthly fishing and zooplankton sampling took place in the pelagic zone of Upper Lake Constance. Gillnet fishing for whitefish was performed with 7-m-high net panels of different lengths and mesh sizes (14, 17, 20, 26, 32, 36, 38, and 40 mm) combined into one 420-m-long fleet. The net panels were set up 2 h before sunset and picked up 1.5 h after sunset, resulting in only 3–4 h of fishing, which prevented significant digestion of fish stomach content. As whitefish depth distribution is temperature dependent, mean fishing depth is changing with the season (Thomas et al. 2010). Surface water temperature during the study period in Lake Constance ranged from 5.1 °C in February 2018 to 22.9 °C in August 2017, when the thermocline extended from 10 to 15 m depth (IGKB 2018, 2020). Therefore, the depth at which nets were set ranged from 5–12 m in May to 32–39 m in January to ensure a sufficient number of samples. Caught fish were handled according to the German Animal Protection Law (§ 4) and the ordinance on slaughter and killing of animals (Tierschutzschlachtverordnung § 13). Immediately afterwards, they were put on ice and transported to the laboratory, where they were weighed (to the nearest 0.01 g and 1 g for sticklebacks and whitefish, respectively), measured (to the nearest 1 mm), and sexed. Stomachs (oesophagus to pylorus) were preserved in 70% EtOH for subsequent content analysis. In parallel to whitefish fishing, zooplankton was sampled. A standard Clarke Bumpus sampler with a 16-cm-wide opening and net mesh of 100 µm was used at different depths (0–10, 10–20, 20–30, 30–40, and 40–60 m), and then all depths were pooled together to obtain an average density for the entire water column (0–60 m). Zooplankton strongly migrates around dusk, and thus sampling was performed twice: immediately after setting up the gillnets and just before picking them up, i.e. before and after sunset (May and July 2017 were without day samples due to issues with the net). The average densities of both (day and night) samplings were used for further analysis.

Sticklebacks were caught by trawling with a 3-m-high and 2-m-wide trawl with a mesh size of 6 mm. The mesh size of the codend was 4 mm. Trawling was conducted after sunset at depths of 0–3, 3–6, 9–12, 15–18, and 21–24 m; the process is described in detail by Gugele et al. (2020). Caught fish were killed with an overdose of clove oil and put into 70% EtOH for stomach (oesophagus to pylorus) content analysis. To ensure comparable data between whitefish and sticklebacks, we aimed to catch both fish species on similar dates. However, due to technical difficulties and weather conditions, sampling dates for both fish species differ by an interval of 0–15 days. For comparing fish diets for each month, the dates July 31, 2017, and November 2, 2017, were denoted as August and October, respectively.

Zooplankton identification

Samples of zooplankton from the lake and stomachs were divided for identification and counted into aliquots of at least 300 individuals using a sedimentation tube with two equal chambers at its bottom. Eight zooplankton taxa were identified: *Eudiaptomus gracilis* Sars, 1862, Cyclopoida Burmeister, 1834, *Bosmina* spp. Baird, 1845, *Daphnia cucullata* Sars, 1862, *Daphnia longispina* O. F. Müller, 1776, *Daphnia galeata* Sars, 1864, *Leptodora kindtii* Focke, 1844, and *Bythotrephes longimanus* Leydig, 1860. Other taxa, including *Diaphanosoma brachyurum* Liévin, 1848 and flying insects or benthic invertebrates, represented less than 0.2% of the stomach content and were thus excluded from the analysis. Damaged zooplankton from stomachs was identified from the remaining fragments as described by Stich and Maier (2006). Data regarding zooplankton species-specific average sizes were obtained from routine zooplankton sampling of the Limnological Institute, University of Konstanz, from May 2017 to April 2018 to complete one full year. If size measurements of certain species were missing (*B. longimanus* and *L. kindtii*), our measurements from the lake samples were used. In both cases, body measurements were done on animals, fixed with 4% formaldehyde (routine sampling) or with 70% EtOH (our measurements).

Data analysis: consumed zooplankton biomass and predator selectivity

Zooplankton dry weight was calculated from species-specific body length, and the dry weight correlations were obtained from multiple authors to gather information for all zooplankton species (Geller 1989; Hälbich 1997; Michaloudi 2005; Bledzki and Rybak 2016). From the counted zooplankton from each fish stomach and the average dry weight of each zooplankton species, the total consumed zooplankton dry weight was calculated for each fish. Dividing this value with fish wet weight yielded the prey:predator ratio (mg/g). The difference in consumed zooplankton dry weight and the prey:predator ratio was compared with the non-parametric Wilcoxon-Mann-Whitney test for two periods during which both fish were present: May 2017 to September 2017 and October 2017 to January 2018.

Fish selectivity for zooplankton species was expressed as the Chesson Index, which considers not only the percentage of prey in the stomach but also in the environment (Chesson 1978): $\alpha_i = (r_i / p_i) / (\sum_{i=1}^m r_i / p_i)$; where r_i = proportion of food item i in the stomach, p_i = proportion of food item i in the environment, m = number of food items in the environment. In our case, m was considered the average number of every zooplankton species per m^3 in the entire sampling depth profile (0–60 m) and was calculated from the average values of the first (day) and second (night) samplings. Values that were equal to zero (i.e. no prey species present) were excluded from the Chesson Index calculation. The index values vary between 0 and 1, where $\alpha > 1 / m$ indicates a preference, and $\alpha < 1 / m$ indicates avoidance of prey species by the predator.

Seasonal Bray-Curtis similarities

Non-metric multidimensional scaling (NMDS) ordination plots based on Bray-Curtis similarities was used to identify seasonal changes in the diet of sticklebacks and whitefish. All analyses were conducted in PRIMER (v.7.0.13, PRIMER-e, Quest Research Limited, Albany, New Zealand). Stomach content data was fourth-root transformed and averaged for each time point (sampling month), and a Bray-Curtis resemblance matrix was created. Time points with less than five individuals were omitted from the analysis. Subsequently, NMDS was performed with 1000 repeats (Kruskal fit scheme = 1, minimum stress = 0.01; Clarke and Warwick 2001).

In order to compare the seasonal patterns of the diet composition between the two fish species, stomach content data of five individuals were pooled (for each species and time point) and subsequently standardized (by total) to improve comparability between the two species. Pooling of five individuals was done to decrease the number of zero values in each category of prey species (Hourston et al. 2004). An overview of the number of replicates for each time point, season, and species after pooling is available in Suppl. material 1: table S2. The data was then transformed (square-root), and a Bray-Curtis resemblance matrix was created. Next, averages were bootstrapped based on species and season ($n = 33$ bootstraps per group, minimum $\rho = 0.99$, bootstrap regions = 95%) and plotted in an NMDS orientation plot (1000 repeats, Kruskal fit scheme = 1, minimum stress = 0.01). Analysis of similarities (ANOSIM) was used to test for significant differences between the diets of whitefish and sticklebacks in each season (one-way, 999 permutations; Clarke 1993). If ANOSIM revealed statistically significant differences between sticklebacks and whitefish in a season, a similarity percentages procedure (SIMPER) was used to identify the contribution of individual prey species to the differences between the two fish species (one-way, 70% cut-off; Clarke 1993).

As both adult and first-year-of-life (0+) sticklebacks were sampled in July and September of 2017, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was used to examine whether the stomach content differed between the two age groups. A Bray-Curtis resemblance matrix (Clarke et al. 2006) was created from the dataset ($n = 15$ adults, $n = 22$ 0+ fish), and PERMANOVA (two-way, 999 permutations, sums of squares: type III (partial), unrestricted permutation of raw data) was conducted, taking into account the factors “age”, “time point”, and their interaction (“age \times time point”). The month of August was excluded from the analysis as only 0+ sticklebacks were caught. In case of statistically significant differences between 0+ and adult sticklebacks, the analysis of seasonal changes in diet and patterns between sticklebacks and whitefish was repeated excluding 0+ sticklebacks. For this purpose, stomach content data of four individuals were pooled (for each fish species and sampling point), standardized (by total), and analysed as described above.

Beta regression and prey-specific index of relative importance

The dependence of the relative abundance in the stomach on \log_{10} transformed relative abundance *in situ* was analysed for each fish and zooplankton species using beta regression, which is an appropriate regression method when dependent variables range between 0 and 1 (Cribari-Neto and Zeileis 2010). To avoid pseudoreplication, beta regression was performed with median relative abundances of each zooplankton species for each fish species sample on each sampling date. As for NMDS, beta regression analysis was performed with and without 0+ sticklebacks (Fig. 5, Suppl. material 1: fig. S2, respectively).

The importance of each prey taxa for the predator diets was expressed with the prey-specific index of relative importance (%PSIRI). It is similar to the commonly used index of relative importance, which uses occurrence, numerical abundance, and biomass of each prey taxon in predator diets, and provides a balance between all three parameters in calculating the index metric (Cortés 1997; Liao et al. 2001). However, %PSIRI is a recommended replacement due to less erroneous behaviour and more balanced treatment of prey quantity measures (Brown et al. 2012): $\%PSIRI = \%FO_i * (\%PW_i + \%PN_i) / 2$; where $\%FO_i$ = frequency of occurrence (present/not present in stomachs), $\%PW_i$ = prey-specific weight, and $\%PN_i$ = prey-specific number in the predator's diet. $PW_i = \sum \%W_i / n_i$ and $PN_i = \sum \%N_i / n_i$; where $\%W_i$ = prey i weight (relative to all prey weight in an individual stomach), $\%N_i$ = prey i number (relative to all prey number in an individual stomach), and n_i = number of stomachs containing prey i.

Data were analysed and plotted with statistical software R (R Core Team 2018), using the packages tidyverse (Wickham et al. 2019), lubridate (Grolemund and Wickham 2011), Rmisc (Hope 2013), rstatix (Kassambara 2021), ggpubr (Kassambara 2020), and betareg (Cribari-Neto and Zeileis 2010). Bray-Curtis similarities and all related analyses and plotting were done with PRIMER v.7.0.13 and JMP Pro 15.2.1 (SAS Institute).

Data availability

The raw data are available via Zenodo at <https://doi.org/10.5281/zenodo.6523369> (after 1.1.2023).

Results

We analysed the stomach contents of 221 whitefish and 144 sticklebacks obtained from monthly fishing in the pelagic zone of Upper Lake Constance between April 2017 and May 2018. Caught whitefish were 180–461 mm long (42–898 g), and sticklebacks were 20–81 mm long (0.05–7.3 g). From July to September, we identified two size groups (0+ and 1+ and older) of sticklebacks. The group of smaller sticklebacks

(0+) measured 20–30 mm in length in July, increased in size through summer, and merged with the group of older sticklebacks in the very beginning of November. The contribution of this group (0+) to all sampled sticklebacks was 55% in July, 100% in August, and 68% in September (Fig. 1A, Suppl. material 1: table S1). Diets could be compared from May 2017 to January 2018, when both fish species were caught in sufficient quantities, i.e. at least 16 each, except for May and January, when only five sticklebacks were caught (see Suppl. material 1: table S1). Both fish species foraged almost exclusively on pelagic zooplankton. Only 18 and 15 whitefish' and sticklebacks' stomachs, respectively, contained other prey (e.g. Diptera imago, Chironomidae pupae, roe) and from these only 4 and 8 whitefish' and sticklebacks' stomachs, respectively, contained prey items typical for the littoral or benthic zone (e.g. Chironomidae larvae or *Gammarus* sp.). Only two sticklebacks' stomachs contained larger amounts ($n = 11$ and $n = 74$) of this type of food.

Consumed prey biomass

From May to September, whitefish had a significantly higher total dry weight consumption per fish than sticklebacks (whitefish, $n = 80$; sticklebacks, $n = 100$, median difference = 12.0 mg, $W = 385$, $p < 0.0001$). However, from October to January, sticklebacks surpassed whitefish in zooplankton consumption (whitefish, $n = 64$; sticklebacks, $n = 76$, median difference = 1.03 mg, $W = 3687$, $p < 0.0001$) (Fig. 1B). In terms of dry weight consumption per weight of fish, sticklebacks consumed more in both mentioned periods (whitefish, $n = 80$; sticklebacks, $n = 100$, median difference = 1.02 mg/g, $W = 6912$, $p < 0.0001$, and whitefish, $n = 64$; sticklebacks, $n = 76$, median difference = 0.53 mg/g, $W = 4596$, $p < 0.0001$ respectively) (Fig. 1C).

Interspecific and intraspecific seasonal diet variability

The smallest crustacean zooplankton in the lake was *Bosmina* spp., followed by copepods, daphniids and both predatory zooplankton species (Fig. 2A). The lowest dry weight had cyclopoid copepods and the highest had *B. longimanus* (Fig. 2B). Small zooplankton had the highest relative abundance, especially copepods, which dominated throughout most of the season (Fig. 2C). However, when larger prey was available their contribution to the fish diet was low. Zooplankton contribution to the diet of each fish individual differed, resulting in high interspecific and intraspecific variability in whitefish and stickleback diets throughout the year (Fig. 2D, E). Both fish diets followed the trend of absolute abundance of zooplankton species. In May 2017, they consumed mostly *Bosmina* spp., which was by far the most abundant species in this month. In summer, they consumed larger and more diverse prey like *B. longimanus*, *L. kindtii* and *D. longispina*. In winter, when other prey was less abundant, they relied mostly on copepods and *D. longispina* (Fig. 3A). From May 2017 to January 2018, the selection of *B. longimanus* strongly prevailed in both fish (Fig. 3B).

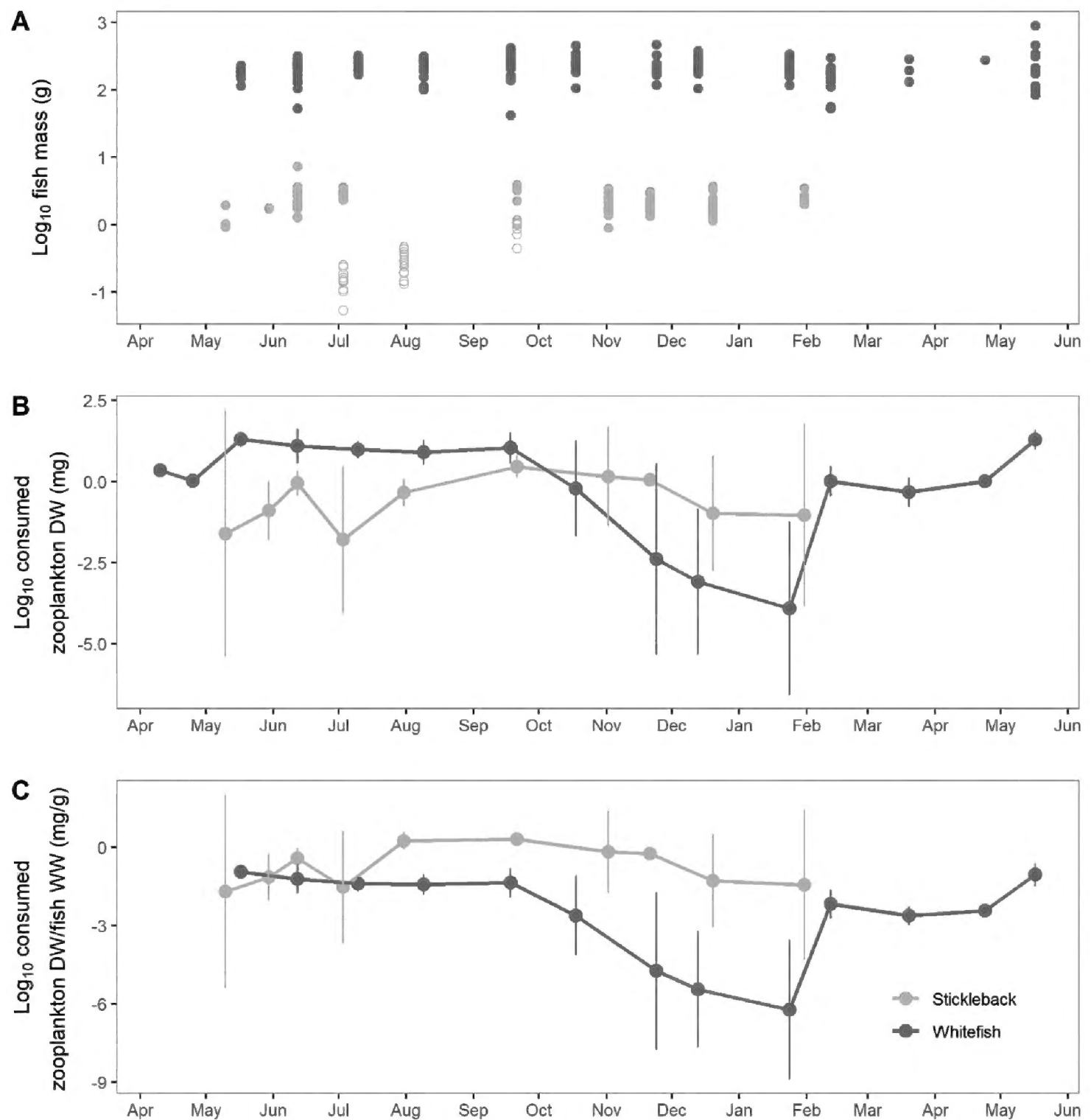


Figure 1. Seasonal changes of whitefish and sticklebacks between 2017 and 2018. Fish mass (empty dots represent sticklebacks that can be identified as a separate, 0+ group; **A**), zooplankton dry weight (DW) consumption (average \pm 1 SD; **B**) and zooplankton DW consumption per gram of fish wet weight (WW) (average \pm 1 SD; **C**). Note that sticklebacks were caught from May until January and that in (**A**), (**C**) values for April are missing due to missing measurements of whitefish weight. Large SD values in some months are due to small sample numbers, non-feeding, or a mixture of 0+ and 1+ fish in the case of July sticklebacks (see Suppl. material 1: table S1).

The results of NMDS indicate seasonal changes in the diets of sticklebacks and whitefish (Fig 4A, B). The stress values of the NMDS ordination plots were 0.1 and 0.09 for sticklebacks and whitefish, respectively. The seasonal changes in the diets of both fish species were compared with bootstrapped averages (Fig. 4C). The stress value of the NMDS ordination plot was 0.14. ANOSIM revealed statistically significant dif-

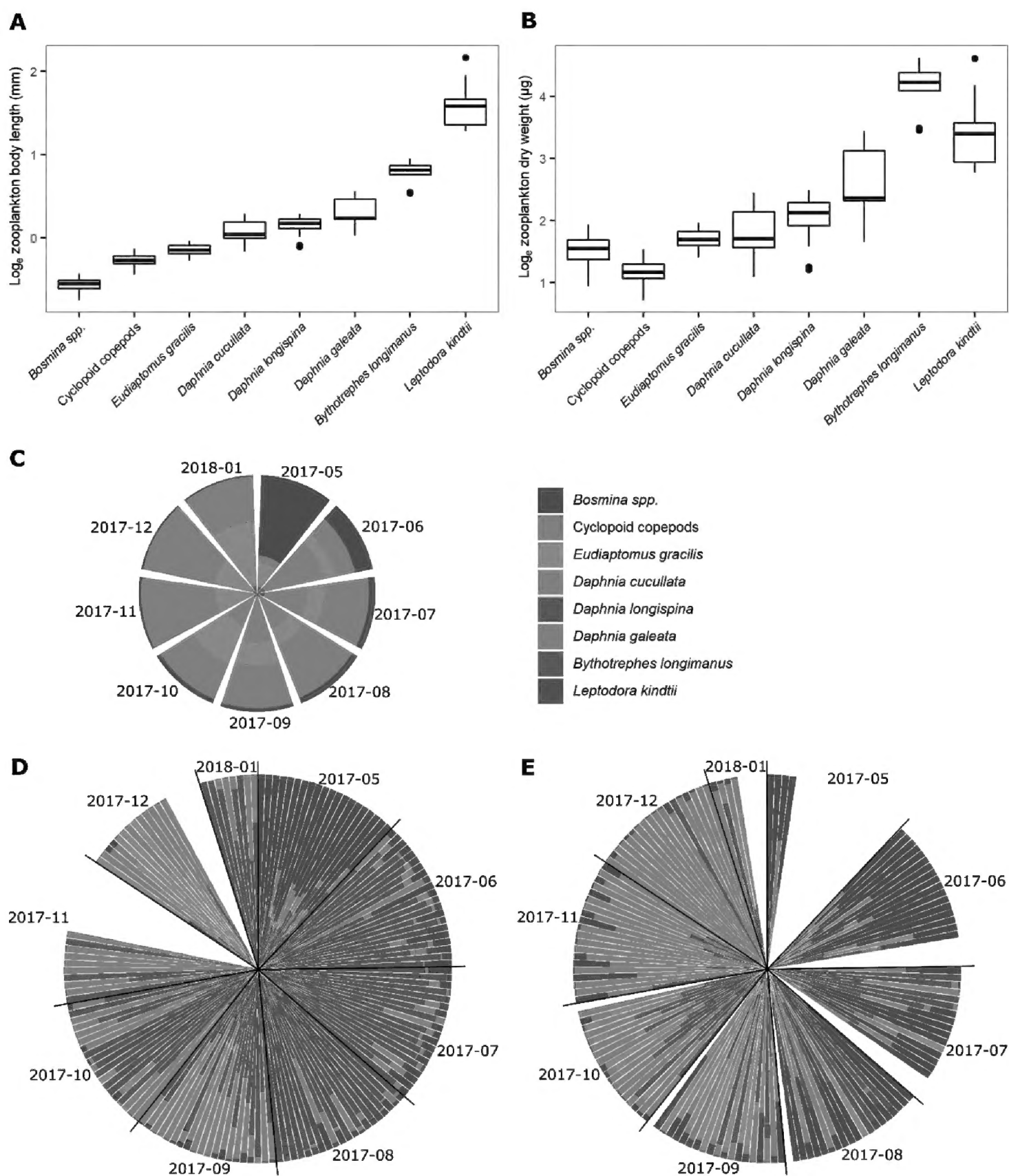


Figure 2. Crustacean zooplankton taxa of Lake Constance and their contribution to fish diets. Zooplankton average body size (**A**), dry weight (**B**), seasonal composition (depth: 0-60 m; **C**) and seasonal contribution to diet of individual whitefish (**D**) and sticklebacks (**E**). Empty slots represent missing data (fish not caught or fish with empty stomachs).

ferences between species ($R = 0.549$, $P = 0.001$). Comparing diets of sticklebacks and whitefish per season with the posthoc tests revealed statistically significant differences in summer and winter (see Suppl. material 1: table S3). However, when 0+ sticklebacks were excluded from the analysis, statistical differences remained only in winter (see Suppl. material 1: fig. S1). The similarity percentages procedure revealed an average

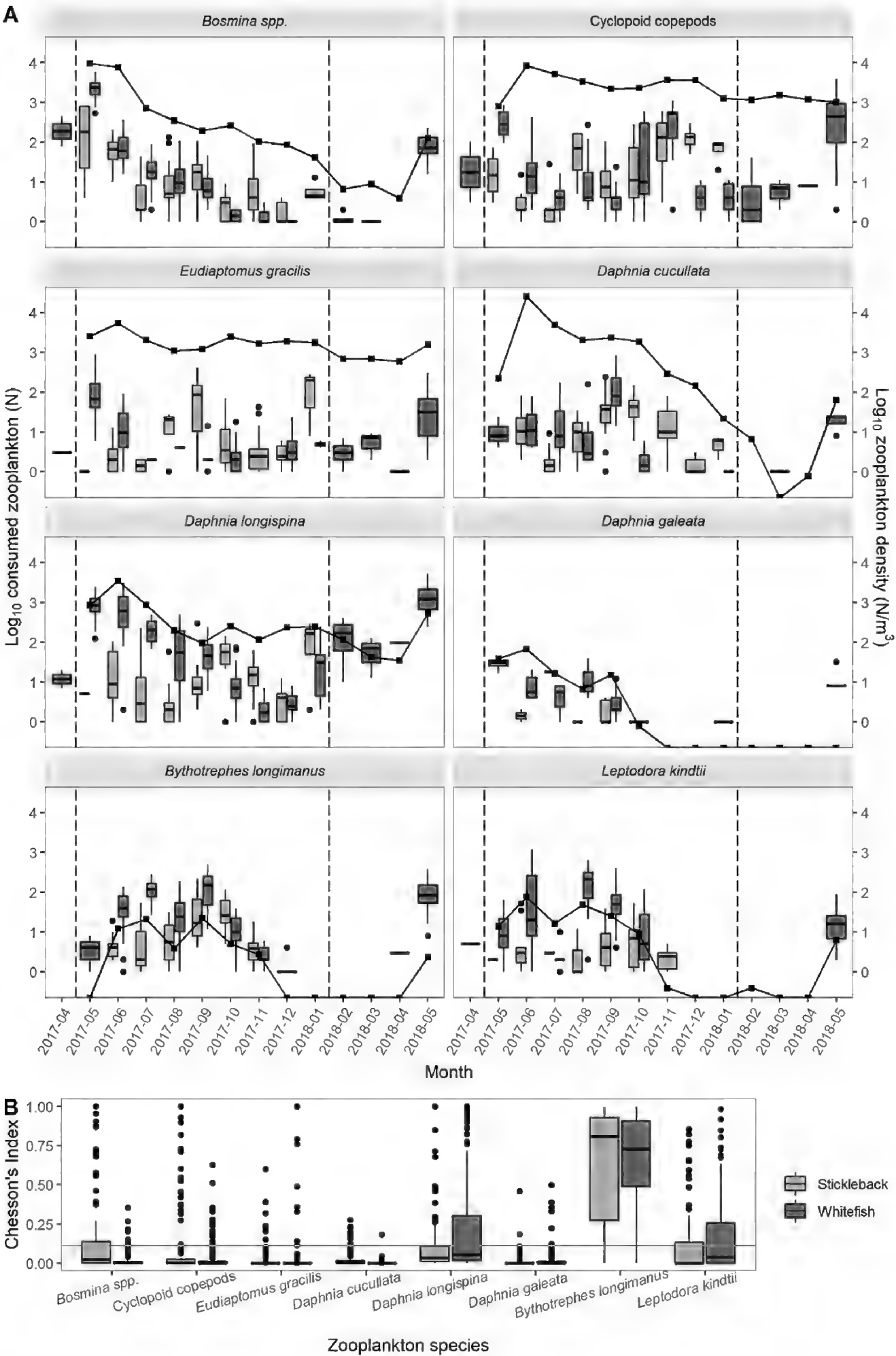


Figure 3. Zooplankton consumption by whitefish and sticklebacks. Seasonal zooplankton consumption (**A**) and Chesson's prey selectivity index from May 2017 to January 2018 (**B**). Lines with black squares represent average zooplankton density (N/m³) in the lake (depth: 0–60 m). The period in which our samples contained both fish species is delimited by dashed vertical lines. Zooplankton is ordered from the smallest to the largest species. Chesson's Index values above and below the red line ($\alpha = 1/m$) represent preference and avoidance, respectively, for each zooplankton species over the compared period.

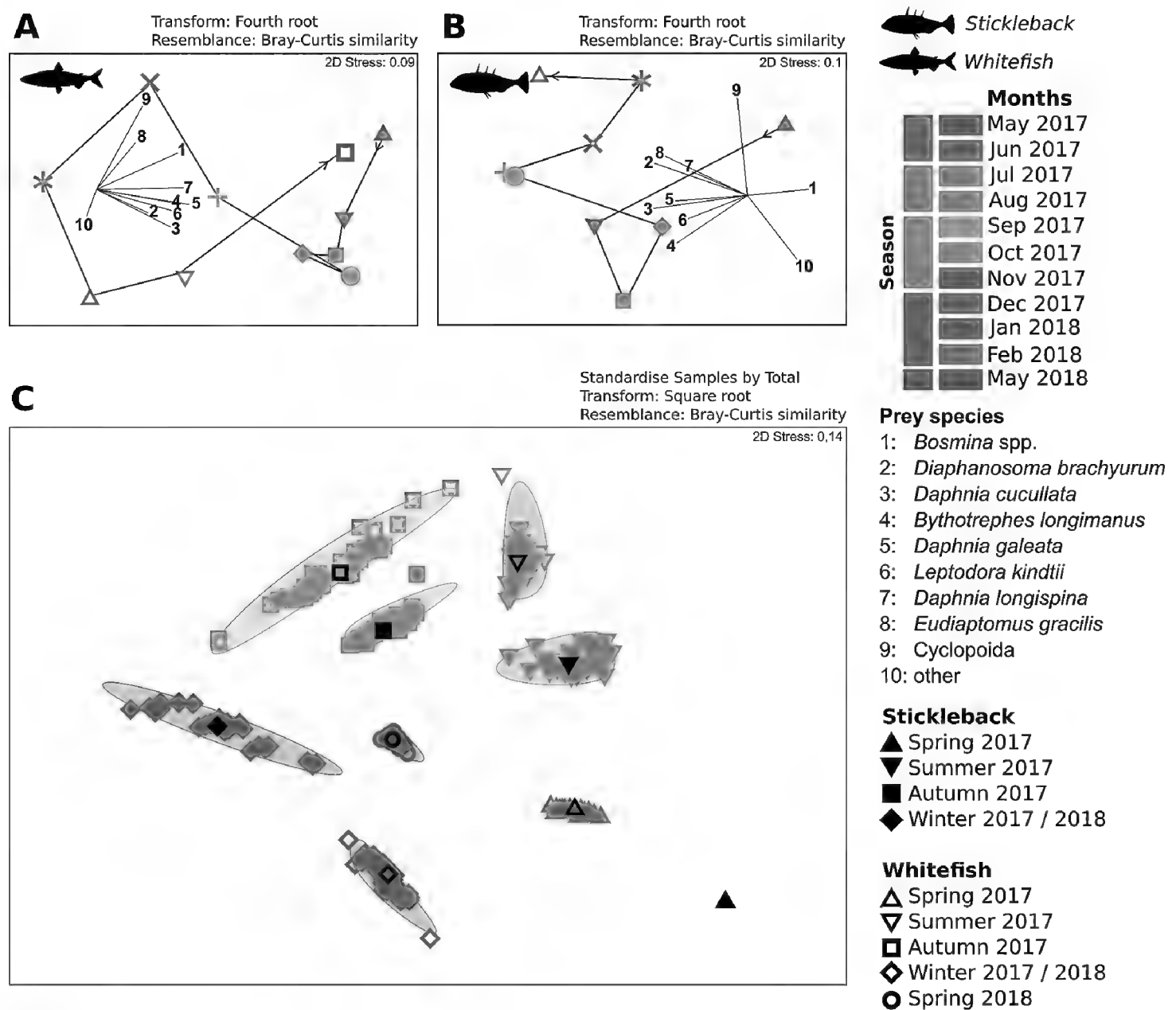


Figure 4. Seasonal changes in the number of consumed prey species for sticklebacks and whitefish in Lake Constance. Non-metric multidimensional scaling (NMDS) ordination plots for whitefish (**A**) and stickleback (**B**) data based on Bray-Curtis similarities. An NMDS ordination plot of bootstrapped averages for both species (**C**). Vectors indicate the direction and strength of individual prey species on orientation (Pearson correlation).

dissimilarity between sticklebacks and whitefish of 46.97%. Cyclopoida and *D. longispina* contributed most to the dissimilarity, with 20.81% and 18.73%, respectively (see Suppl. material 1: table S4). Likewise we found statistically significant differences in diet composition between the two age groups of sticklebacks ($df = 1$, pseudo $F = 6.5429$, $p = 0.001$), the two time points ($df = 1$, pseudo $F = 9.8253$, $p = 0.001$), and their interactions ($df = 1$, pseudo $F = 4.5848$, $p = 0.001$).

Feeding on large or abundant prey

Fish intensively preyed on large zooplankton species (*D. longispina*, *B. longimanus*, and *L. kindtii*) already at low relative abundances, whereas they consumed smaller zooplankton species only when these species were the most dominant prey (Figs 2, 5).

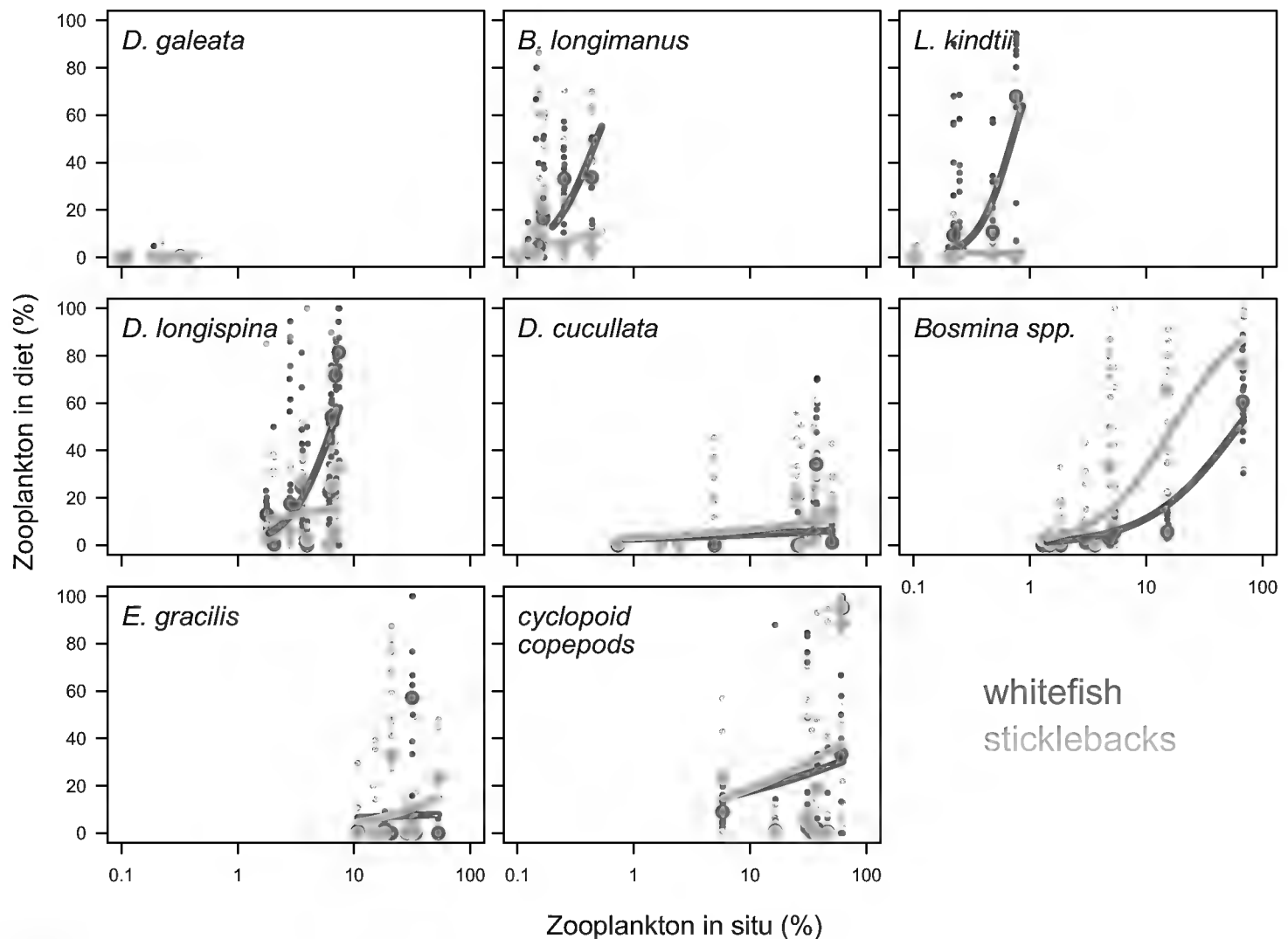


Figure 5. Relationships between the percentages of zooplankton species in diets versus *in situ* for whitefish and sticklebacks. Small dots represent the diet contributions in individual fish, and large dots represent the median diet contribution at the various sampling dates. The lines show the fits from beta regression based on the median diet contributions.

The consumption of all species, except *E. gracilis*, cyclopoid copepods and *D. galeata*, increased with increasing relative densities of the species *in situ*. *Bosmina* spp. was significantly more consumed by sticklebacks than by whitefish. A significant interaction between fish species and relative zooplankton density was observed for *D. longispina* and *L. kindtii* due to strong increases in diet with increasing relative abundances of these zooplankters for whitefish, but not for sticklebacks (Fig. 5, Suppl. material 1: table S5).

The importance of various prey species in fish diets

Prey-specific indices of relative importance (%PSIRI) (Fig. 6) indicate that *D. galeata* was never important in the fish diets (%PSIRI always < 0.5%). *D. cucullata* was (except in September) more important prey for sticklebacks than for whitefish, whereas *Bosmina* spp. was very important for both fish species in May (%PSIRI > 58%), and for sticklebacks also during summer (%PSIRI > 21% until the August). Cyclopoid copepods had the highest %PSIRI values for both fish species in November and

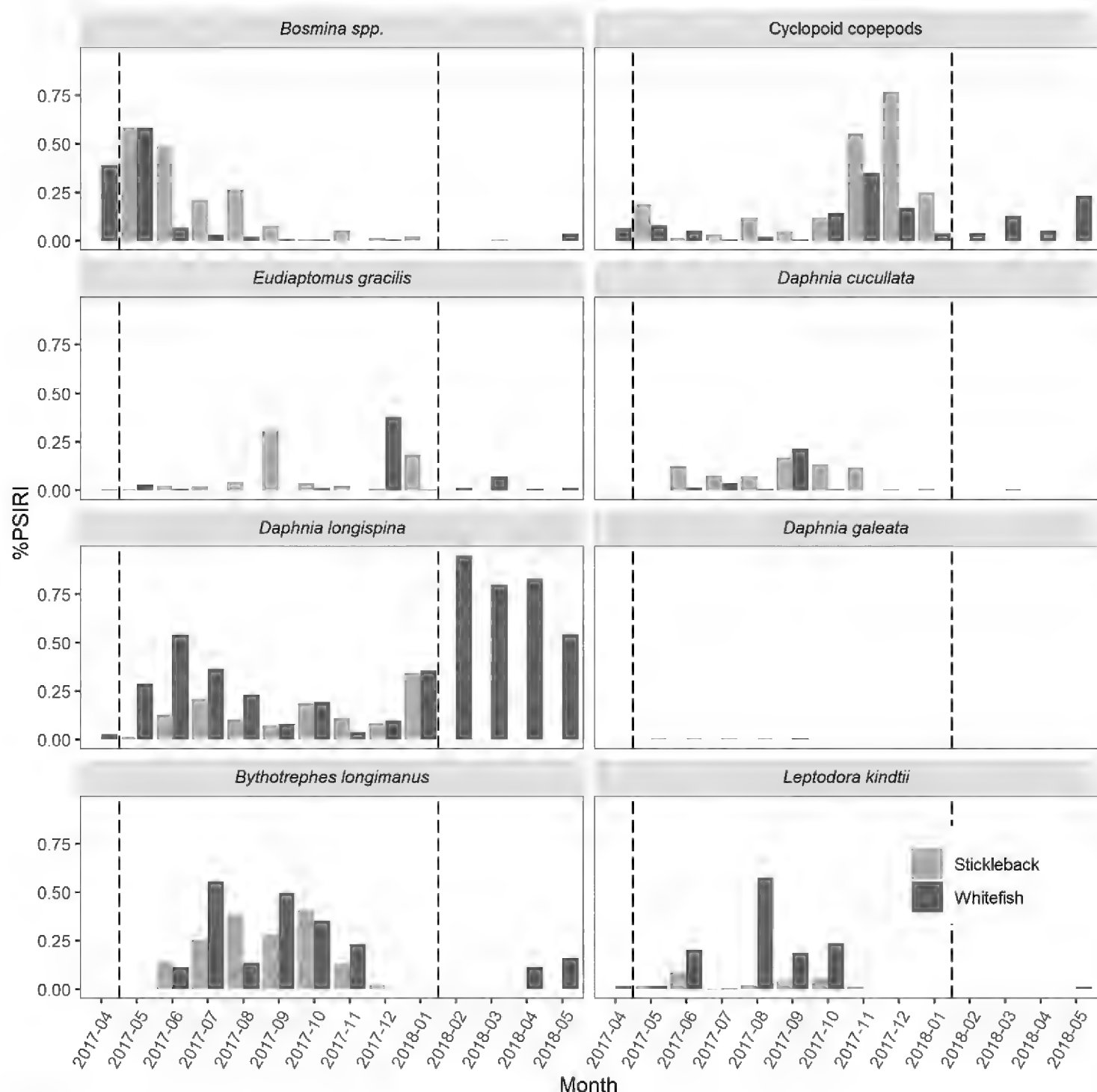


Figure 6. The seasonal prey-specific index of relative importance (%PSIRI) for each zooplankton species in whitefish and sticklebacks. The period in which our samples contained both fish species is delimited by dashed vertical lines. Zooplankton species are ordered from the smallest to the largest species.

December (%PSIRI between 17% and 77%), whereas *E. gracilis* had the highest %PSIRI values for whitefish in December (%PSIRI = 38%) and for sticklebacks in September (%PSIRI = 31%). Among all zooplankton species, *D. longispina* had the most persistent %PSIRI values for all seasons (%PSIRI always > 1%) and was especially important prey for whitefish in winter (%PSIRI up to 95%). *L. kindtii* had high %PSIRI values only for whitefish in some summer and autumn months (%PSIRI up to 57%). The most important prey for both fish species from May 2017 to January 2018 was *B. longimanus* (with an average %PSIRI values in this period of 27% for whitefish and 23% for sticklebacks). Throughout this period, average %PSIRI values above 10% were observed for *D. longispina* for both fish species, *Bosmina* spp. and Cyclopoida for sticklebacks, and *L. kindtii* for whitefish. The importance of *D. longispina* strongly

increased (%PSIRI = 40%) when the entire year was considered (May 2017 to April 2018; data available only for whitefish) instead of the compared period (see Suppl. material 1: table S6).

During the compared period (May 2017 to January 2018), both large predatory species (*B. longimanus* and *L. kindtii*), *Bosmina* spp., and Cyclopoida appeared in approximately equal numbers and occurrences in the whitefish diet, whereas *D. longispina* dominated in numeric contribution and *B. longimanus* in biomass contribution (see Suppl. material 1: fig. S3A). In the stickleback diet, *B. longimanus* contributed the most in biomass, whereas Cyclopoida contributed the most in number (see Suppl. material 1: fig. S3B).

Discussion

Invasive species often present a threat to native species because of competition for the same food resources. This study demonstrates that invasive sticklebacks, which weigh 100-fold less than native whitefish, had a higher food consumption per body weight and even consumed more food per individual fish in some autumn and winter months. Despite many morphological, behavioural, and size differences between the two fish species, the number of consumed prey species overlapped during most of the year and differed only in winter; in summer, their diets differed only when 0+ sticklebacks were included in the analysis. Moreover, similar zooplankton species were of high importance for both fish species, with rare, large, and conspicuous *B. longimanus* being the most preferred and important prey. This could lead to food competition, especially for highly selected prey items during periods of limited resources.

Consumed prey biomass

As assumed according to Kleiber's law, sticklebacks had higher consumption per body weight than whitefish. Surprisingly, in late autumn and winter, sticklebacks consumed even more zooplankton per individual. To the best of our knowledge, our study is the first one to demonstrate that in the winter season, small fish consumed more food than the large cold-water fish species. During this time, zooplankton density generally dropped, large zooplankton species, e.g. *L. kindtii* and *B. longimanus*, disappeared and adult whitefish consumed less prey. With lower temperatures, body metabolism drops, and many fish species reduce their feeding activities (Johnston and Dunn 1987). During longer periods of hibernation or low food intake, larger and fatter organisms have an advantage over smaller organisms because of their higher ratio between reserve size and basal metabolism (Ultsch 1989; van Deurs et al. 2011). However, regardless of size, fish mortality is lower if they acquire food during winter (Thompson et al. 1991; Heermann et al. 2009; Geissinger et al. 2021), especially for cold-water fish species, which are adapted to be active at low temperatures (Sullivan 1986). Whitefish are cold-water fish species (Kottelat and Freyhof 2007) that also actively feed during winter if there is enough food (Hayden et al. 2022).

Winter anorexia was shown mostly for fish for which the risk of predation is high. In such cases, fish prefer to reduce their activity and hide, unless they risk death from starvation (Farley et al. 2011; van Deurs et al. 2011). In the pelagic zone of Lake Constance, piscivorous fish are rare (Alexander and Vonlanthen 2016). Thus, feeding is predicted to be a more successful strategy, provided more energy is gained from prey than is spent to catch prey. As shown in an aquaria study, small fish feed on small zooplankton at higher rates than large fish (Ogorelec et al. 2022). It is unclear whether larger fish have lower catching abilities or ignore small prey due to the relatively smaller energy income per small zooplankton.

Interspecific and intraspecific seasonal diet variability

Besides relatively high amounts of consumed prey in certain months, sticklebacks also consumed similar prey species as whitefish throughout most of the year. Winter was an exception, during which whitefish relied on larger available prey (*D. longispina* and *E. gracilis*) or stopped feeding (see above), whereas sticklebacks continued to consume a large amount of smaller but more abundant cyclopoid copepods. Differences in summer were only observed when the predominant 0+ sticklebacks were included in the analysis: although these sticklebacks also preferred large zooplankton, they consumed a lower proportion of large zooplankton than adults (e.g. from July to September, *B. longimanus* represented 33% and 4% of prey abundance in the diets of adult and 0+ sticklebacks, respectively). Of note, 0+ whitefish were not present in our samples due to their efficient avoidance of gillnetting (Sandlund and Næsje 1989) and trawling. However, they have similar feeding rates as those of 0+ sticklebacks (Ogorelec et al. 2022), which suggests that including 0+ whitefish in the analysis would decrease, rather than increase observed dietary differences between species.

The interspecific differences might have been obscured because of certain methodological and biological issues. i) The intraspecific diet variability was high, which is in line with reports on zooplankton patchiness (Wiebe 1971) and fish specialisation on a few prey species that visually match the fish searching image concept (Lazzaro 1987). The small sample numbers in some months thus might have resulted in poor representations of the entire population. ii) Although fish sampling was planned to occur on as similar dates as possible, dates differed by up to 15 days because of lack of manpower and poor weather conditions. Especially in springtime, zooplankton composition can change within this time frame (Seebens et al. 2013), most likely affecting fish diets. iii) Finally, gillnet depths were selected according to the preferences of harder-to-catch whitefish, whereas trawling for sticklebacks always occurred at the same depths. To account for the high variability within species and between sampling dates and efficiencies, monthly data were grouped into seasons. Assessing the depths of occurrence and their relation to prey selection was beyond the scope of this study, however, as both fish species occupy similar water column depths (Thomas et al. 2010; Gugele et al. 2020), the difference in fish diets between species should not be affected much by depth and temperature.

Feeding on large or abundant prey

Despite their large size differences, both fish species equally favoured large and conspicuous zooplankton, especially *B. longimanus*. This species is among the most preferred prey by whitefish both in Lake Constance (Becker and Eckmann 1992) and in other pre-Alpine and Alpine lakes (Mookerji et al. 1998; Gerdeaux et al. 2002; Müller et al. 2007). Although many authors have reported that small fish avoid *B. longimanus* because of its spine (Barnhisel 1991; Barnhisel and Harvey 1995; Jarnagin et al. 2000), we observed that *B. longimanus* was not only consumed by sticklebacks longer than 25 mm but was also their most preferred prey (this preference further increased with increasing fish size). Although *L. kindtii* had the largest body size among the sampled zooplankton, it was not the most selected, probably due to its transparency and, consequently, low conspicuousness. By contrast, the most selected species, *B. longimanus*, has a large and conspicuous eye, which is important for attracting fish (Lazzaro 1987).

Although large prey was positively selected, it was not the most abundant (especially predatory *L. kindtii* and *B. longimanus*) and therefore not necessarily the most consumed. In the spring of 2017, when densities of other zooplankton taxa were much lower, fish consumed high amounts of *Bosmina* spp., even though it was the smallest crustacean zooplankton species in the lake. In this year, densities of *Bosmina* spp. were exceptionally high (almost as high as the maximum observed during eutrophic conditions; Straile and Geller 1998), but already next year their numbers were lower in the lake and in the fish diets. Similar results of whitefish preying on *Bosmina* spp. were also found in Lake Lucerne (Mookerji et al. 1998). Other studies also showed that predation on smaller zooplankton is high only when the abundance of larger, and thus preferred, zooplankton is low (Ivlev 1961; Lazzaro 1987). When prey appears at very high densities, the energy and time required for searching for prey are significantly reduced (Holling 1959). The profitability of prey is its energy value subtracted by the predator's energy requirements to find and consume prey per time unit (Sinervo 2007). Thus, the abundance of *Bosmina* spp. might compensate for its presumably low energy value by reducing the energy required for searching. Furthermore, *Bosmina* spp. is not as evasive as copepods, thus predators have a higher attack efficiency and lower handling time (Lazzaro 1987).

The importance of various prey species in fish diets

Although *B. longimanus* represented less than 0.1% of the number of all zooplankton in the lake, it was the most important prey and contributed the highest biomass to the diets of both fish species from late spring to autumn. It was absent in colder months, and thus its importance in the annual whitefish diet was surpassed by *D. longispina*, which was the largest zooplankton species during winter. Among zooplankton, *Daphnia* is one of the most important and most selected prey items for fish because of its abundance, size, nutritional value, and low evasiveness (Lazzaro 1987). In our study, this high importance was indicated only for larger *D. longispina*, but not for *D. cucullata*, even though the latter has become the most abundant cladoceran since 2016 (IGKB 2020). Likewise in a mesocosm study,

both, whitefish and sticklebacks strongly suppressed *D. longispina*, whereas *D. cucullata* was less affected by fish predation (Ogorelec et al. 2021). In general, *D. cucullata* is less prone to fish predation because of its smaller size and narrower body (Gliwicz 2001). Even though it did not migrate and was present mostly in the epilimnion, which should make it more vulnerable to fish predation compared to migratory *D. longispina*, it contributed more to the whitefish diet only in September, when its density was 25-fold higher than that of *D. longispina*. The third daphniid species in our samples, *D. galeata*, was not present in Lake Constance in pre-eutrophic times (Auerbach et al. 1924); however later, in eutrophic times, it became very abundant in the lake and important in the whitefish diet (Becker and Eckmann 1992). Our study was performed during the oligotrophic state of the lake and revealed a low abundance and importance of *D. galeata* for fish, indicating the reversibility of its role (which became insignificant again) after re-oligotrophication.

Bosmina spp. and cyclopoid copepods were of high importance in fish diets in spring and autumn, respectively. Although they had the lowest mass among crustacean zooplankton (Fig. 2B), their occurrence and numbers were high in fish diets (see Suppl. material 1: fig. S3) when other prey was mostly absent, which resulted in high %PSIRI values for those months. Although larger and also very abundant, *E. gracilis* was not an important prey item, which is probably linked to its evasiveness (Lazzaro 1987). Its low contribution to fish diets was also observed in many other lakes (Mookerji et al. 1998; Mehner et al. 2008). The higher consumption of *L. kindtii* by whitefish than by sticklebacks could be due to different foraging strategies. Whereas sticklebacks use the hover search strategy, whitefish rely more on the swim search strategy (Ogorelec et al. 2022), which enables searching through a larger volume of water and (in the case of sinusoidal swimming) can help detect prey due to changing light conditions that increase the contrasts (and shadows) of inconspicuous prey, e.g. *L. kindtii* (Jarolim et al. 2010).

The effects of sticklebacks on zooplankton and planktivorous fish

Comparing our study with previous findings regarding whitefish in Lake Constance in eutrophic times and without sticklebacks in the pelagic zone (Becker and Eckmann 1992) indicates that the whitefish diet has declined quantitatively (decreased numbers of zooplankton in stomachs) and qualitatively (smaller zooplankton species in stomachs). As whitefish growth is most strongly related to standing stock biomass followed by phosphorus concentrations (Thomas and Eckmann 2007), both a new food competitor and re-oligotrophication can decrease whitefish growth (Deweber et al. 2022). Similar diets do not directly indicate competition; when resources are abundant, diets may overlap to any degree without competition for resources. However, when resources are limited, diet similarity and competition can be directly related (Sale 1974), which is most probably the case in oligotrophic Lake Constance.

An aquarium experimental study demonstrated no differences in the feeding rates between co-occurring sticklebacks and 0+ whitefish, whereas similar-sized sticklebacks had larger feeding rates than those of whitefish (Ogorelec et al. 2022). However, these findings cannot account for the advantage of the swim-search strategy of whitefish when not spatially limited. In the field, at least large whitefish seem to have some

advantage, as they can search through larger volumes of water, have a larger stomach capacity, and can thus consume higher numbers of large zooplankton species when they are abundant. However, when food becomes scarcer (in late autumn and winter), the competition presumably increases. Studies from Lake Constance have shown that in the eutrophic and oligotrophic state of the lake, most whitefish fed abundantly also during winter (Auerbach et al. 1924; Becker and Eckmann 1992). However, our current study has shown that now, after the stickleback invasion, many whitefish (up to 50%; see Suppl. material 1: table S1) had empty stomachs during winter.

In contrast to (pre-eutrophic) studies of whitefish (Auerbach et al. 1924) and recent studies of sticklebacks (Roch et al. 2018), our study did not find any fish larvae in the diets of either fish. This suggests that fish larvae might only be important prey for sticklebacks in the littoral zone or on specific occasions, e.g. during whitefish larvae stocking (Roch et al. 2018). Due to rapid evolution, sticklebacks may now diverge into groups occupying littoral, pelagic or profundal habitats (Hudson et al. 2021), albeit, up to now only subtle genetic differences between individuals occupying different habitats were observed (Dahms et al. 2022). Our study showed that the pelagic sticklebacks have already specialised in feeding on zooplankton. All sticklebacks were caught offshore (>100 m water depth), and the proportion of fish that had any littoral/benthic prey in their stomachs was low (5.5%).

High numbers of sticklebacks (Alexander and Vonlanthen 2016), and their high biomass consumption throughout most of the year are very likely exerting strong effects on the zooplankton community. In contrast to whitefish, whose multiple generations inhabit the pelagic zone throughout the entire year (Eckmann et al. 2007; DeWeber et al. 2021), sticklebacks shift habitat in May, migrating to the littoral zone to spawn (Gugele et al. 2020). This might reduce the predation pressure on pelagic zooplankton in a lake dominated by sticklebacks during this period. However, in summer, adult sticklebacks returned to the pelagic zone together with large numbers of rapidly growing 0+ sticklebacks. Therefore, predation on large zooplankton increased, especially in September when sticklebacks tend to appear in vast densities (Gugele et al. 2020) and, according to our data, consume high amounts of zooplankton. In winter, when zooplankton densities in the lake were low, stickleback consumption remained high and thus presumably strongly affected zooplankton community abundance and structure. Stickleback invasion is thus the most probable reason for recent zooplankton changes, especially the increased proportion of small zooplankton species, e.g. *D. cucullata* (IGKB 2020).

To date, few reports have investigated sticklebacks invading the pelagic zone and interacting with other pelagic fish or zooplankton. The exception is the Baltic Sea, where numerous studies tried to reveal the causes and consequences of stickleback increase (Olin et al. 2022). One study showed that sticklebacks are potential competitors for herring and sprat due to similar diets and prey selection (Jakubavičiute et al. 2016), whereas others showed that sticklebacks suppress native fish by preying on their larvae (Ljunggren et al. 2010; Byström et al. 2015; Eklöf et al. 2020). This is in accordance with the findings of Roch et al. (2018) and our current study for Lake Constance, which together demonstrate that sticklebacks most likely suppress whitefish populations and force predator

shifts by consuming prey and juveniles of native predators. Due to the rapid colonisation of new areas and the invasiveness of sticklebacks (Fang et al. 2018; Hudson et al. 2021), this small fish could present a large threat to indigenous aquatic species.

Conclusions

This study has contributed to our understanding of the diets of both whitefish and sticklebacks, and has provided insights into the interplay between both small and large as well as native and invasive fish species. It has shown that sticklebacks successfully fed all year round, also in winter, when some whitefish stopped feeding. Owing to their small size, sticklebacks also have lower absolute metabolic demands than whitefish, and thus their energy acquisition in winter is distinctively higher. Further bioenergetics research is needed to evaluate whether larger fish are less successful in capturing small and evasive zooplankton or whether they ignore this prey due to negative profitability. Such information could provide important insights into global invasions of small pelagic fish species. When 0+ sticklebacks and the winter season were excluded, no seasonal differences in the number of consumed prey species were observed. Furthermore, our findings do not indicate that specialised whitefish are more selective predators than sticklebacks. Similar prey preference and importance, especially for conspicuous *B. longimanus* and other large prey, indicate a high probability of interspecific competition between both fish species. The high numbers and effective and persistent feeding of invasive sticklebacks, as indicated in this study, affect not only whitefish populations, but presumably also zooplankton communities. This may explain the appearance and numerical dominance of small and less preferred zooplankton species, e.g. *D. cucullata*, and the reduced growth and yield of whitefish after the invasion of sticklebacks. As Lake Constance is similar to many other pre-Alpine lakes in this region, potential invasions of pelagic stickleback populations could cause drastic and irreversible changes in the food webs and ecosystem functioning of such lakes.

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Supplementary material I

Supplementary data

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Data type: docx file

Explanation note: **table S1.** Number of sampled fish and fish with empty stomachs for each month. In May 2017, sticklebacks were caught on two occasions: 3 sticklebacks on the 10th and 2 sticklebacks on the 30th of May. Sticklebacks were also classified into a separate first-year-of-life (0+) group according to their sizes. **table S2.** Number of replicates for each sampling time point after pooling five samples for sticklebacks and whitefish. **table S3.** Differences between whitefish and stickleback diets in each season. **table S4.** The contribution of individual prey species to the differences between stickleback and whitefish diets (similarity percentages procedure; one-way, 70% cut-off). **table S5.** Results of beta regression relating relative zooplankton consumption to relative zooplankton density, fish species, and the interaction between relative density and fish species (see Fig. 5). **table S6.** Summary of the prey-specific index of relative importance (%PSIRI) for both fish predators for the compared period (May 2017 to January 2018). Asterisks represent calculations made for whitefish for the entire year (May 2017 to April 2018). **figure S1.** Seasonal changes in the diets of sticklebacks (excluding 0+) and whitefish in Lake Constance. **figure S2.** Whitefish and stickleback (excluding 0+ fish) relative consumption of zooplankton species depending on the proportional density of zooplankton species. **figure S3.** Annual averages of the three parameters (% occurrence, body weight and relative abundance (% number)) of the prey-specific index of relative importance for A) whitefish and B) sticklebacks.

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